Clin. exp. Immunol. (1987) **70**, 562–569

Serum levels of interferons in patients with systemic lupus erythematosus

T. KIM, Y. KANAYAMA, N. NEGORO, M. OKAMURA, T. TAKEDA & T. INOUE *The First Department of Internal Medicine, Osaka City University Medical School, Osaka, Japan*

(Accepted for publication 16 June 1987)

SUMMARY

Serum levels of alpha (α) and gamma (γ)-interferons (IFN) were measured in 30 patients with untreated systemic lupus erythematosus (SLE) with a solid-phase, sandwich immunoradiometric assay using specific monoclonal antibodies. The serum levels of α-IFN were higher in patients with SLE than in normal subjects, and correlated with the clinical activity index ($r_s = 0{\cdot}60$, $P < 0{\cdot}01$), but not with renal histological activity. The serum level of α-IFN correlated with the serum level of immune complexes ($r = 0{\cdot}46$, $P < 0{\cdot}01$) and the number of peripheral lymphocytes inversely ($r = -0{\cdot}49$, $P < 0{\cdot}01$). Serum γ-IFN levels were also higher in patients with SLE than in control subjects, but no correlations were found between it and either clinical activity, renal histological activity or various laboratory parameters. Serum levels of both α-IFN and γ-IFN were higher in SLE patients with erythema than in those without. These results suggested that serum levels of α-IFN were more closely related to clinical activity of SLE than were those of γ-IFN, and that peripheral lymphocytes were probably not the source of the elevated serum IFN-α concentration.

Keywords α-interferon γ-interferon systemic lupus erythematosus serum levels radioimmunoassay monoclonal antibody

INTRODUCTION

Human interferons (IFN) are classified into three groups on the basis of their antigenic properties (Interferon Nomenclature Committee, 1980). α-Interferon (α-IFN) is produced mainly by leucocytes in response to a variety of viral and nonviral stimuli and is stable at pH 2. β-Interferon (β-IFN) is synthesized predominantly by fibroblast-like cells and, to a much lesser extent, by leucocytes, and is also acid-stable. γ-Interferon (γ-IFN) is released by lymphocytes following exposure to mitogens or specific antigens (Epstein, 1979). γ-IFN is inactivated by incubation at pH 2 and is generally more heat-labile than either α-IFN or β-IFN (Valle *et al.*, 1975). γ-IFN is thought to participate in a variety of immunoregulatory events, for example, in human mononuclear phagocytes inducing the expression of DR-determinants (Basham & Merigan, 1983; Kelly, Fiers & Strom, 1984), and enhancing both cellular antigen presentation to lymphocytes (Zlotnick *et al.*, 1983) and natural killer cell activity (Targan & Stebbing, 1982). γ-IFN has also been shown to enhance a variety of lymphocyte functions, including expression on T lymphocytes of interleukin 2 receptors (Johnson & Farrar, 1983) and on B lymphocytes of DR-determinants (Wong *et al.*, 1983), as well as causing inhibition of suppressor T lymphocyte activity (Knop *et al.*, 1982).

Correspondence: Yoshiharu Kanayama MD, 1st Department of Internal Medicine, Osaka City University Medical School, 1-5-7, Asahimachi, Abeno-ku, Osaka 545, Japan.

Hooks *et al.* (1979) were the first to detect IFN activity in the circulation of patients with SLE, and concluded that it was γ-IFN due to its inactivation at pH 2. Subsequent studies, however, showed it to be an unusual acid-labile α-IFN (Preble *et al.*, 1982; Hooks *et al.*, 1982; Ytterberg & Schnitzer, 1982), thus leaving the presence of γ-IFN in the sera of these patients uncertain.

Recently, solid-phase, sandwich immunoradiometric assays for human α-IFN and γ-IFN using specific monoclonal antibodies have been developed (Chang *et al.*, 1984; Scott *et al.*, 1985), enabling detection of very low serum INF levels. We employed them to detect α-IFN and γ-IFN levels in serum samples from untreated SLE patients, and compared the data with SLE disease activity, including renal histology, several immunoserological parameters, and clinical features.

MATERIALS AND METHODS

Patient population. Serum samples were taken from 30 patients with a clinical diagnosis of SLE between 1983 and 1986. All patients met the 1982 revised criteria for the classification of SLE (Tan *et al.*, 1982). None of the patients had received steroids or immunosuppressive drugs at the time of

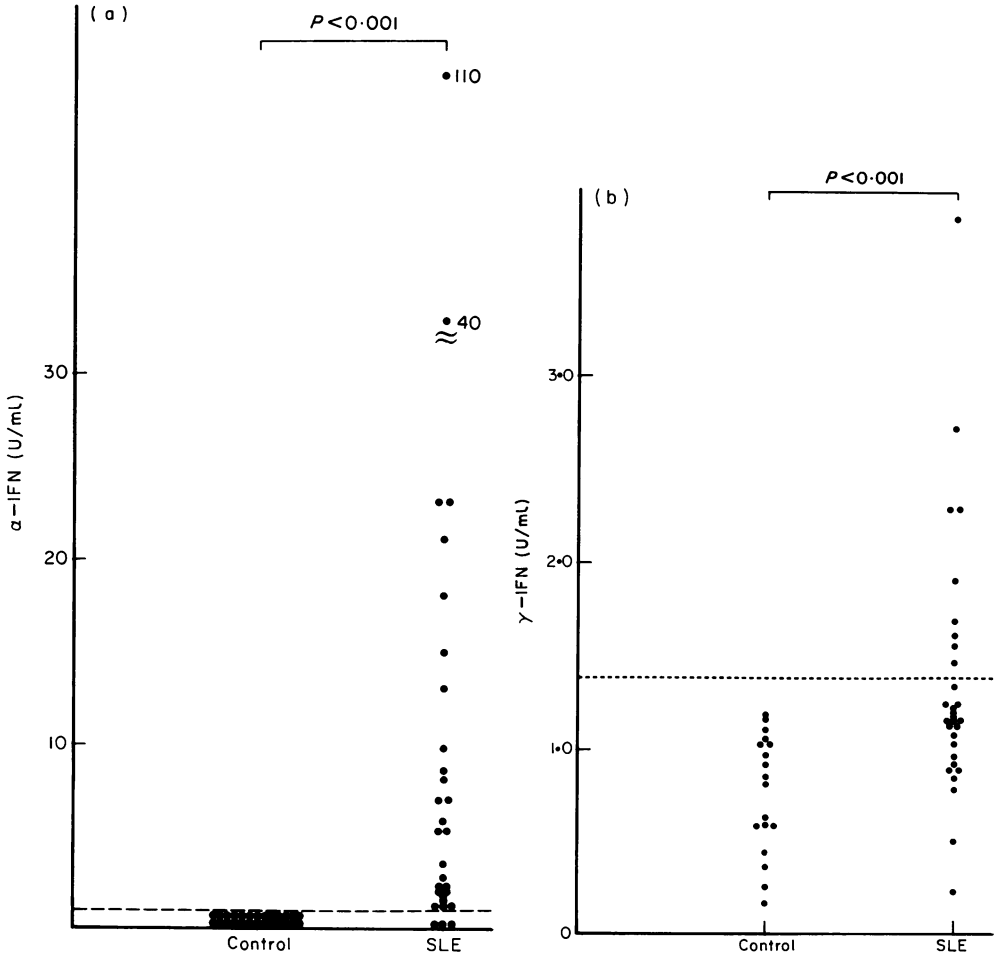

Fig. 1. Serum levels of α- and γ-interferons in patients with SLE ($n=30$) and in normal subjects ($n=26$). The dotted line indicates the upper limit of normal levels.

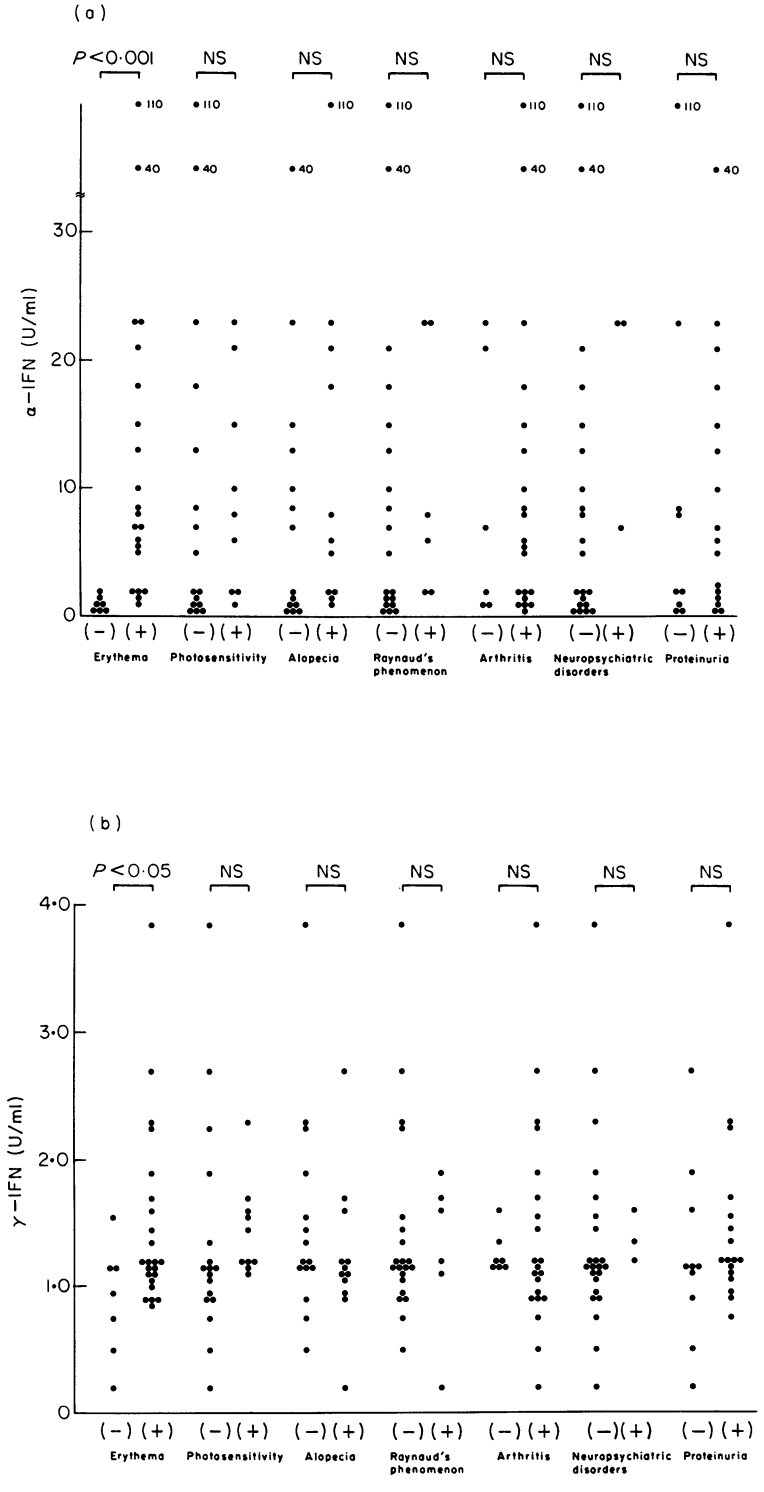

Fig. 2.(a). Comparison of α-interferon serum levels in SLE patients with or without each clinical manifestation **(b).** Comparison of γ-interferon serum levels in SLE patients with or without each clinical manifestation.

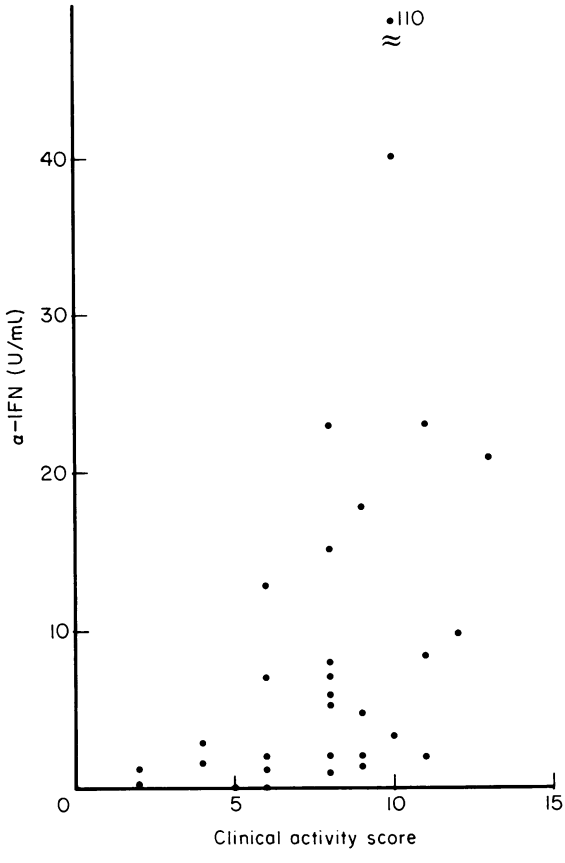

Fig. 3. Correlation of α-IFN serum levels with clinical activity index. $r_s = 0{\cdot}60$; $P < 0{\cdot}01$.

serum collection. These sera were stored at −80°C until use. Renal biopsies were undergone on the 27 patients either at the pretreatment stage or within 10 days of initiation of therapy.

Interferon assays. Serum sample γ-IFN levels were determined by the solid-phase, sandwich immunoradiometric assay described by Chang *et al.* (1984) using the commercially available kit (Centcor, Malvern, PA, USA). In brief, B1 monoclonal antibody coated polystyrene beads, used as a solid-phase immunoadsorbent for human γ-IFN, were incubated with 200 μl of test samples in an assay tray at room temperature for 2 h without shaking. The beads were washed with water and incubated with 200 μl of a tracer solution containing 10^5 ct/min ^{125}I-B3 monoclonal antibody at room temperature for 2 h. After washing, the beads were assayed for ^{125}I in a gamma counter. Partially purified natural γ-IFN (Yip *et al.*, 1982) diluted with heat-inactivated pooled normal human serum was used as an assay standard.

α-IFN levels in the same serum samples were measured by an immunoradiometric assay kit using a monoclonal antibody against α-IFN (Boots-Celltech Diagnostics, Slough, UK). The procedures were essentially the same as those described above. Samples were measured in duplicate.

Measurement of anti-DNA antibodies and circulating immune-complexes. Levels of double-stranded DNA antibodies were measured by a modification of Farr's method (Holian *et al.*, 1975). Circulating immune complexes were evaluated quantitatively by the C1q binding assay (Hay, Nineham & Roitt, 1976).

Clinical disease activity index for SLE. To determine the patient's disease activity at the time of blood sampling, the scoring system used for the SLE clinical disease activity index, which was described by Morimoto *et al.* (1982), was employed.

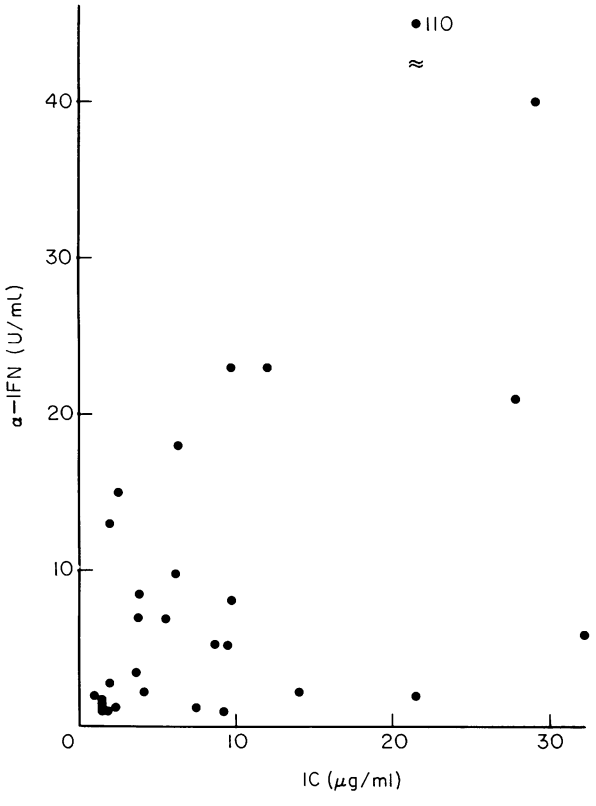

Fig. 4. Correlation of α-IFN serum levels and serum levels of immune complexes. $r=0{\cdot}46$; $P<0{\cdot}01$.

Renal histological activity index. Biopsy specimens from 27 patients were fixed in buffered formalin solution, embedded in paraffin and stained with either haematoxylin-eosin, periodic acid-Schiff or Masson's trichrome stains. Histological features were scored according to the renal histological activity indices described by Austin *et al.* (1983).

Normal controls. Serum samples were taken from healthy laboratory personnel, and were assayed in the same way as those taken from patients. The upper limit for normality was set at 2 standard deviations above the mean values obtained for the healthy controls.

Statistics. Statistical analysis was done using Student's *t*-test, Wilcoxon signed-rank test, linear regression analysis and the nonparametric rank order correlation method of Spearman, where appropriate.

Values were expressed as mean ± s.d.

RESULTS

Levels of both α-IFN and γ-IFN in serum samples from the SLE patients were higher than in those from the healthy subjects (11·37 ± 20·76 U/ml vs less than 1 U/ml (below the sensitivity limit of the assay) for α-IFN $P<0{\cdot}001$; 1·35 ± 0·70 U/ml vs 0·76 ± 0·31 U/ml for γ-IFN, $P<0{\cdot}01$) (Fig. 1). α-IFN levels being elevated in 27 and γ-IFN in nine of the 30 patients. Both were greatly elevated in serum from SLE patients who had cutaneous erythema (14·6 ± 22·9 vs 3·1 ± 2·4 U/ml for α-IFN $P<0{\cdot}001$; 1·5 ± 0·7 vs 0·9 ± 0·4 U/ml for γ-IFN, $P<0{\cdot}05$). On the other hand, neither were further elevated in serum from SLE patients with additional clinical symptoms of arthritis, proteinuria, photosensitivity, neuropsychiatric disorders, alopecia and Raynaud's phenomenon (Fig. 2).

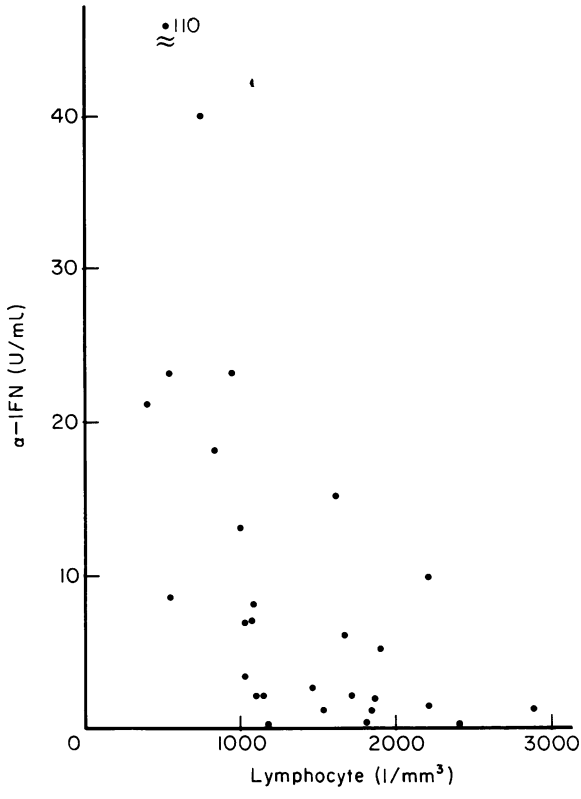

Fig. 5. Correlation of α-IFN serum levels and the number of peripheral lymphocytes. $r=0{\cdot}49$; $P<0{\cdot}01$.

Table 1. Spearman's correlation coefficients between the clinical activity index and various laboratory parameters

	α-INF	γ-INF	Anti-DNA antibody	Immune complex	CH50	C3	C4
r_s	0·60	0·31	0·56	0·35	0·73	0·58	0·65
P	<0·01	NS	<0·01	NS	<0·01	<0·01	<0·01

The serum level of α-IFN correlated with SLE clinical disease activity ($r_s=0{\cdot}60$, $P<0{\cdot}01$) (Fig. 3), but not significantly with renal histological activity ($r_s=0{\cdot}26$). It was also correlated with immune complex serum levels ($r=0{\cdot}46$, $P<0{\cdot}01$) (Fig. 4) and the number of peripheral lymphocytes ($r=-0{\cdot}49$, $P<0{\cdot}01$) (Fig. 5), but showed correlations with neither serum levels of anti-DNA antibodies ($r=0{\cdot}03$), total haemolytic complement ($r=-0{\cdot}22$), C3 ($r=-0{\cdot}34$) and C4 ($r=-0{\cdot}18$) nor the number of peripheral leucocytes ($r=-0{\cdot}29$). γ-IFN serum levels correlated with none of the above. A correlation between serum α-IFN levels and serum γ-IFN levels was found ($r=0{\cdot}51$, $P<0{\cdot}01$).

Table 1 shows the correlation coefficients between the clinical disease activity index and the various laboratory parameters, including α-IFN and γ-IFN serum levels.

DISCUSSION

Although we found that the serum levels of α-IFN and γ-IFN in patients with SLE were higher than those in normal subjects, the elevation of γ-IFN level was small. Still higher γ-IFN and γ-IFN serum levels were observed in SLE patients with cutaneous erythema, but those with other clinical manifestations, including arthritis, neuropsychiatric disorders, alopecia, proteinuria, Raynaud's phenomenon and photosensitivity showed no such additional increases. These findings suggest that serum α-IFN and γ-IFN levels could be useful in defining SLE patient subsets.

The significant correlation of the level of α-IFN with the SLE clinical disease activity, comparable with that of serum anti-DNA antibodies (Table 1), is consistent with the results reported by other investigators (Hooks *et al.*, 1979; Preble *et al.*, 1982; Ytterberg & Schnitzer, 1982).

The α-IFN found in the serum of SLE patients is mostly known to be acid-labile (Preble *et al.*, 1982; Hooks *et al.*, 1982), and is thought to be produced by blood mononuclear cells (Balkwill *et al.*, 1983), especially large granular lymphocytes concurrently with γ-IFN (Fischer & Rubinstein, 1983).

Lymphocytes from SLE patients are poor producers of IFN in culture (Neighbour & Grayzel, 1981; Tsokos *et al.*, 1982; Strannegard, Hermodsson & Westberg, 1982; Preble *et al.*, 1983; Sibbitt *et al.*, 1985), and Preble *et al.* (1983) found an inverse correlation between SLE patient IFN serum levels and the amount of IFN produced by SLE patient-derived lymphocytes *in vitro*. We found a significant inverse correlation between the number of peripheral lymphocytes and the serum levels of α-IFN, suggesting that this α-IFN was not produced by lymphocytes in the circulation, but originated from stimulated lymphocytes located either within a lymphoid organ, or more likely, at a localized site of lymphocyte infiltration.

Schattner (1983) proposed a role for interferon in inducing lymphopenia in SLE, a proposal supported by our observation of an inverse correlation between α-IFN serum levels and the number of peripheral lymphocytes.

Out of a number of immunological laboratory parameters, serum levels of α-IFN correlated with that of immune complexes, suggesting an immune complex involvement in the stimulation of α-IFN production as was previously suggested by Fujibayashi, Hooks & Notkins, (1975).

γ-IFN is thought to participate in a variety of immunoregulatory events, its administration to NZB/WF1 mice, for example, clearly exacerbating the nephritis that occurs spontaneously in these animals (Engelman *et al.*, 1979). However, we found serum γ-IFN levels correlated with neither SLE clinical activity nor with renal histological activity, and the highest serum level detected was less than 4 U/ml, generally regarded as 'negative' in the conventional bioassay. Thus, failure of serum γ-IFN levels to correlate with disease activity may be due to the low levels being insufficient to elicit biological reactions *in vivo*. However, the possibility cannot be excluded that localized γ-IFN levels in either lymphoid organs or at a site of lymphocyte infiltration were high enough to elicit potent immunoregulatory effects without a spillover into the serum.

Recently, Czernielewski & Bagot (1986) suggested that *in vivo* keratinocyte HLA-DR antigen expression during different dermatological inflammatory disorders originated from lympho-epidermal interactions and local γ-IFN production. This suggestion may be relevant to our observations that γ-IFN serum levels were higher in SLE patients with erythema than in those without.

REFERENCES

AUSTIN, H.A., MUENZ, L.R., JOYCE, K.M., ANTONOVYCH, T.A., KULLICK, M.E., KLIPPEL, J.H., DECKER, J.L. & BALOW, J.E. (1983) Prognostic factors in lupus nephritis: contribution of renal histologic data. *Am. J. Med.* **75,** 382.

BASHAM, T.Y. & MERIGAN, T.C. (1983) Recombinant interferon-γ increases HLA-DR synthesis and expression. *J. Immunol.* **130,** 1492.

BALKWILL, F.R., GRIFFIN, D.B., BAND, H.A. & BEVERLEY, P.C.L. (1983) Immune human lymphocytes produce an acid-labile α-interferon. *J. exp. Med.* **157,** 1059.

CHANG, T. W., MCKINNEY, S., LIU, V., KUNG, P.C., VILCEK, J. & LE, J. (1984) Use of monclonal antibodies as sensitive and specific probes for biologically active human γ-interferon. *Proc. natn Acad. Sci. USA.* **81,** 5219.

CZERNIELEWSKI, J.M. & BAGOT, M. (1986) Class II

MHC antigen expression by human keratinocytes results from lympho-epidermal interactions and γ-interferon production. *Clin. exp. Immunol.* **66,** 295.

ENGELMAN, E.G., SONNENFELD, G., BANTING, G., MERIGAN, T. & MCDEVITT, H.O. (1979) Type II interferon accelerates glomerulonephritis and death in NZB/NZW mice. *Arthritis Rheum.* **22,** 606.

EPSTEIN, L.B. (1979) The comparative biology of immune and classical interferons. In *Biology of the Lymphokines.* (S. Cohen, E. Pick & J. Oppenheimer. p. 443. Academic Press, Inc., New York.

FISCHER, D.G. & RUBINSTEIN, M. (1983) Spontaneous production of interferon-γ and acid-labile interferon-α by subpopulations of human mononuclear cells. *Cell. Immunol.* **81,** 426.

FUJIBAYASHI, T., HOOKS, J.J. & NOTKINS, A. (1975) Production of interferon by immune lymphocytes exposed to herpes simplex virus-antibody complexes. *J. Immunol.* **115,** 1191.

HAY, F.C., NINEHAM, L.J. & ROITT, I.M. (1976) Routine assay for the detection of immune complexes of known immunoglobulin class using solid phase C1q. *Clin. exp. Immunol.* **24,** 396.

HOLIAN, J., GRIFFITHS, I.D., GLASS, D.N., MAINI, R.N. & SCOTT, J.T. (1975) Human anti-DNA antibody: reference standards for diagnosis and management of systemic lupus erythematosus. *Ann. Rheum. Dis.* **34,** 438.

HOOKS, J.J., MOUNTSOPOULOS, H.M., GEIS, S.A., STAHL, N.I., DECKER, J.L. & NOTKINS, A.L. (1979) Immune interferon in the circulation of patients with autoimmune disease. *New Engl. J. Med.* **301,** 5.

HOOKS, J.J., JORDAN, G.W., CUPPS, T., MOUNTSOPOULOS, H.M., FAUCI, A.S. & NOTKINS, A.L. (1982) Multiple interferons in the circulation of patients with systemic lupus erythematosus and vasculitis. *Arthritis Rheum.* **25,** 396.

INTERFERON NOMENCLATURE COMMITTEE (1980) Interferon nomenclature. *Nature* **286,** 110.

JOHNSON, H.M. & FARRAR, W.L. (1983) The role of a gamma interferon-like lymphokine in the activation of T-cells for expression of interleukin-2 receptors. *Cell. Immunol.* **75,** 154.

KELLY, V.E., FIERS, W. & STROM, T.B. (1984) Cloned human interferon-γ, but not interferon-β or -α, induces expression of HLA-DR determinants by fetal monocytes and myeloid leukemic cell lines. *J. Immunol.* **132,** 240.

KNOP, J., STREMMER, R., NEUMANN, C., DEMAEYER, E. & BILLIAU, A. (1982) Interferon inhibits the suppressor T-cell response of delayed-type hypersensitivity. *Nature* **296,** 757.

MORIMOTO, C., SANO, H., ABE, T., HONMA, M. & STEINBERG, A.D. (1982) Correlation between clinical activity of systemic lupus erythematosus and the amounts of DNA/anti-DNA antibody immune complexes. *J. Immunol.* **129,** 1960.

NEIGHBOUR, P.A. & GRAZEL, A.I. (1981) Interferon production in vitro by leucocytes from patients with systemic lupus erythematosus and rheumatoid arthritis. *Clin. exp. Immunol.* **45,** 576.

PREBLE, O.T., BLACK, R.J., FRIEDMAN, R.M., KLIPPEL, J.H. & VILCEK, J. (1982) Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leucocyte interferon. *Science* **216,** 429.

PREBLE, O.T., ROTHKO, K., KLIPPEL, J.H., FRIEDMAN, R.M. & JOHNSTON, M.I. (1983) Interferon-induced 2–5 adenylate synthetase *in vivo* and interferon production *in vitro* by lymphocytes from systemic lupus erythematosus patients with and without circulating interferon. *J. exp. Med.* **157,** 2140.

SCHATTNER, A. (1983) Lymphopenia in systemic lupus erythematosus: possible role of interferon. *Arthritis Rheum.* **26,** 1415.

SCOTT, G.M., ROBINSON, J.A., SECHER, D.S., ASHBURNER, C.M. & ABBOTT, S.R. (1985) Measurement of interferon from in-vitro stimulated lymphocytes by bioassay and monoclonal antibody-based immunoassay. *J. gen. Virol.* **66,** 1621.

SIBBIT, W.L. JR, GIBBS, D.L., KENNY, C., BANKHURST, A.D., SEARLES, R.P. & LEY K.D. (1985) Relationship between circulating interferon and anti-interferon antibodies and impaired natural killer cell activity in systemic lupus erythematosus. *Arthritis Rheum.* **28,** 624.

STRANNEGARD, O., HERMODSSON, S. & WESTBERG, G. (1982) Interferon and natural killer cells in systemic lupus erythematosus. *Clin. exp. Immunol.* **50,** 246.

TAN, E.M., COHEN, A.S., FRIES, J.F., MASI, A.T., MCSHANE, D.J., ROTHFIELD, N.F., SCHALLER, J.G., TALAL, N. & WINCHESTER, R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum.* **25,** 1271.

TARGAN, S. & STEBBING, N. (1982) *In vitro* interactions of purified cloned human interferons on NK cells: enhanced activation. *J. Immunol.* **129,** 934.

TSOKOS, G.C., ROOK, A.H., DJEU, J.Y. & BALOW, J.E. (1982) Natural killer cells and interferon responses in patients with systemic lupus erythematosus. *Clin. exp. Immunol.* **50,** 239.

VALLE, M.J., JORDAN, G.W., HAAHR, S. & MERIGAN, T.C. (1975) Characteristics of immune interferon produced by human lymphocyte cultures compared to other human interferons. *J. Immunol.* **155,** 230.

WONG, G.H.W., CLARK-LEWIS, I., MCKIMM-BRESCHKIN, J.L., HARRIS, A.W. & SCHRADER, J.W. (1983) Interferon-γ induces enhanced expression of Ia and H-2 antigens on B-lymphoid, macrophage, and myeloid cell lines. *J. Immunol.* **131,** 788.

YIP, Y.K., BARROECLOUGH, B.S., URBAN, C. & VILCEK, J. (1982) Purification of two subspecies of human γ (immune) interferon. *Proc. natn Acad. Sci. USA.* **79,** 1820.

YTTERBERG, S.R. & SCHNITZER, T.J. (1982) Serum interferon levels in patients with systemic lupus erythematosus. *Arthritis Rheum.* **25,** 401.

ZLOTNICK, A., SHIMONKEVITZ, R.P., GEFTER, M.L., KAPPLER, J. & MARRACK, P. (1983) Characterization of γ-interferon-mediated induction of antigen-presenting ability in P388D1 cells. *J. Immunol.* **131,** 2814.